DEVELOPMENT OF THE POLLEN IN SOME ASCLEPIADACEAE.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XXXII.

T. C. FRYE.

(WITH PLATE XIII)

IT IS well known that in the Cynanchoideae[1] the microspores of each sporangium adhere in a mass, forming what is known as a pollinium. This is true also of some of the Orchidaceae and Leguminosae. Since the three families mentioned are all highly specialized for insect pollination, the adherence of pollen may perhaps be regarded as having no special morphological significance. The Asclepiadaceae are further exceptional in the production of only two sporangia in each stamen. In the Balanophoraceae, Orchidaceae, and Mimosoideae, the number of microsporangia is variable. The large number in some of the Mimosoideae is attributed to the interjection of plates of sterile tissue. In the same way *Lemna minor* is said to form four, the normal number, from a single mass of archesporial tissue.[2] The question arises whether, in the Cynanchoideae, there is any indication of suppression or fusion of sporangia in the earliest stages of their development. More interesting does this become when we recall that in the small group Secamoneae the pollinium in each half anther is paired, but the parts adhere closely.

Little is known about the formation of pollen in the Asclepiadaceae. In the Periplocoideae tetrads are formed, the microspores adhering in groups of four when mature. Of the pollen development in the Cynanchoideae almost nothing is known.

[1] The author follows the classification of Engler and Prantl in *Die natürlichen Pflanzenfamilien.*

[2] CALDWELL, O. W., On the life history of *Lemna minor.* BOT. GAZ. **27**: 37–66. 1899.

Chauveaud[3] claims that the pollen mother cells of Cynanchum divide, but mentions only one division, with reduced number of chromosomes, the pollen grains forming from the daughter cells. Here one is left to infer that a mother cell formed only two microspores, or that there was a second division which escaped his observation. Wille[4] examined *Asclepias speciosa*, but was unable to find tetrads. Strasburger[5] finds no tetrad division in *Asclepias Syriaca*. He finds in the anthers what he terms sporogenous cells, radially elongated, large, and rich in contents. These divide by cross walls into two, and the longest, near the middle of the sporangium, into four. He says the resulting cells are homologous with the pollen mother cells of other plants, and develop into microspores without further division. So far as I have been able to learn Zostera[6] and the Cynanchoideae are the only Angiosperms which are reported to form their pollen without tetrad divisions. It was thought that there were no tetrad divisions in the Cyperaceae until Juel[7] found that the nuclear division occurred, but no walls were formed. The reported different pollen formation in Cynanchum and Asclepias, the presence of tetrads in the Periplocoideae, and the small number of cases of pollen formation without tetrad division, all seem to warrant an investigation of the formation of microspores in the Cynanchoideae.

In the spring of 1901 I undertook the task of looking into the pollen formation of some members of the Asclepiadaceae growing abundantly in the vicinity of Chicago. Those examined were *Asclepias Cornuti*, *A. tuberosa*, *A. phytolaccoides*, *A. incarnata*, *A. verticillata*, *Acerates viridiflora*, and *A. longifolia*.

In *A. Cornuti* I was able to trace the development from the

[3] De la reproduction chez les dompte-venin 41. (Diss.) Paris, 1892.

[4] Ueber die Entwickelungsgeschichte der Pollenkörner der Angiospermen 41. 1886.

[5] Ueber das Wachsthum vegetabilischer Zellhäute. Histologische Beiträge **2**: 80. 1889.

[6] HOFMEISTER, Bot. Zeit. **10**: 121. 1852.

[7] Beiträge zur Kenntniss der Tetradentheilung. III, Die Entwickelung der Pollenkörner bei Carex. Jahrb. f. wiss. Bot. **58**: 649. 1900.

archesporium to the pollen mother cell. The first indication of the formation of sporangia is the increase in length of the hypodermal cells on the inner side of the stamen on each side of its median line, thus forming the archesporium (*fig. 1*). While both sporangia are formed from the same continuous layer, they are separate from their beginning; and there are no indications of four. The tapetum on the dorsal side of the sporangium is formed from the third layer of cells — the one next the archesporium. The archesporial cells elongate at right angles to the inner surface of the stamen and divide transversely, resulting in an inner primary sporogenous, and an outer primary wall layer. The beginning of this division is shown in *fig. 1*. By transverse divisions of the primary wall layer there arise four wall layers (*fig. 3*), the inner one or two of which form the tapetum. The tapetal nature of these cells is already evident in their large nuclei and nucleoli, and their deeply staining contents, before they have completed their division (*fig. 4*). In some places the tapetum is composed of one layer, in others of two. Where there are two the outer is often composed of flattened, the inner of isodiametric cells (*figs. 6* and *14*). While the primary wall layer forms its four layers, the plate of cells dorsal to the sporangium also divides and takes on its tapetal character. The primary sporogenous cells contain large vacuoles, while their nuclei are near the middle (*fig. 4*); but even at this stage of development they grade into ordinary vegetative cells at the outer edge of the sporangium, so that it is difficult to determine just where they cease to be sporogenous cells (*fig. 3*). Enough stages were seen in the other species mentioned to warrant the conclusion that the development of the pollen in them is the same in general as in *A. Cornuti*.

From this point the history was most completely followed in *A. tuberosa*. The primary sporogenous cells become the spore mother cells without further division. This is indicated by the nature of the nuclei and by the form and number of the chromosomes. The size of the nucleus increases, and the chromatin collects in granular tangled threads, which together with

the large nucleolus form the staining contents of the large transparent nuclear vacuole — the synapsis stage of the nucleus (*fig.* 7). This stage of the nucleus was also observed in *Asclepias Cornuti*, *A. phytolaccoides*, *A. verticillata*, and *Acerates longifolia*. At the beginning of mitosis the nucleolus often separates into several smaller nucleoli. The chromatin in *A. tuberosa* breaks into five chromosomes which split longitudinally while scattered in the nuclear vacuole (*fig.* 6). They are very small when split, but become much larger as the nuclear wall and the nucleoli disappear (*fig.* 6). After the disappearance of the nucleoli they are short, thick, and much larger than those in the vegetative cells. The number of chromosomes in the spore mother cells is approximately half that in the vegetative cells (compare *fig.* 6 with *figs.* 8 and 9). The exact number in the vegetative cells I was unable to determine. This reduction of chromosomes occurs in the spore mother cells of other plants. The mitotic phenomena attending the division of the cells referred to as spore mother cells closely agree, then, with those in the spore mother cells of other plants, and do not agree with those in the vegetative cells. This is to me conclusive evidence of their spore mother cell nature.

Closely following this division, often before the cross wall is distinguishable, comes the second, with smaller chromosomes, which results in a row of four daughter cells representing a tetrad (*fig.* 10).

Both the rapid succession of these divisions and the formation of four and only four cells from a mother cell homologize well with tetrad formation. Each mother cell divides; those at the pointed upper end of the sporangium slope outward and downward, as shown in *fig.* 5, thus permitting the division of all the cells without great difference in size. The lower end of the sporangium is rounded, and hence the cells there are not greatly modified. These divisions I have no doubt are the ones Strasburger saw in *A. Syriaca*, and this creates a doubt in my mind whether the mother cells near the ends of the sporangia formed only two microspores, as the report would lead one to believe.

This formation of microspores in a row, while not the usual tetrad form, is not peculiar to the Asclepiadaceae. The cells are reported in other arrangements in *Typha latifolia*,[8] *Orchis mascula*,[9] Juncus,[10] and some members of the Periplocoideae.[11]

The cells now become isodiametric, irregular, and crowded. Adjusting themselves to each other they soon lose all indication of their origin as a row of four. The protoplasm becomes filled with short coarse threads which stain readily and obscure the other cytoplasmic structures (*fig. 11*). The walls then thicken very rapidly. This is by the deposition of a layer from within the cells, as is evident later when intercellular spaces appear. When a mature pollinium is cut and squeezed the walls separating the microspores split, and the microspores are squeezed out whole. Where a microspore is in contact with a pollinium wall it separates from it an inner layer (*fig. 16, x*). This suggests that most of the pollinium wall is of a different origin from the walls of the microspores. The nucleus divides after the walls are thickened, forming the generative and tube nuclei (*fig. 13*). The tube nucleus is much the larger and denser. The generative nucleus moves to the side of the cell (*fig. 14*), and is cut off by a wall of considerable thickness (*fig. 15*). The tapetum gradually disintegrates, leaving the pollinium loose in the anther, except for its attachment to the caudicle by its upper end. After the walls begin to thicken the thready protoplasm collects in masses within the cells (*fig. 12*). The masses disappear about the time of the formation of the generative and tube nuclei, and dense fusiform bodies composed of one or more strands, and of unknown nature, make their appearance. In *A. verticillata* they were short, thick, and smaller than in the other species. Their origin may be connected with the thready and dense protoplasm just preceding their appearance. They

[8] SCHAFFNER, J. H., Development of the stamens and carpels of *Typha latifolia*. BOT. GAZ. **24**: 93. 1897.

[9] WILLE, Ueber die Entwickelungsgeschichte der Pollenkörner der Angiospermen 39. 1886.

[10] *Ibid.* 42.

[11] SCHUMANN, Engler and Prantl, Nat. Pflanzenfam. 4^2: 196. 1895.

remained and were seen even in the pollen tubes. The pollen of all the species studied contained them, but they were most abundant in *A. Cornuti*. The tests for calcium oxalate, carbonate, phosphate, and sulfate, and for silicon and starch failed to reveal their nature. They stain like chromatin.

A stage like that in *fig. 16* was frequently found, and seems to indicate the fragmentation of the tube nucleus, although the series about it was not close enough to justify a definite conclusion.

Germination always bursts the pollinium along its outer edge, at the point where the diameter is greatest (*fig. 17*). A cross section shows the pollinium wall to be thinnest there. Germination of the microspores was observed in *A. Cornuti*, *A. tuberosa*, and *A. incarnata*.

SUMMARY.

The development of the sporangia in the Asclepiadaceae studied is the same in general as in other plants, while there are no indications of the phylogenetic history of the reduction in number. The primary sporogenous cells without further division become the pollen mother cells. The latter divide each into four with the usual phenomena accompanying tetrad division, but through mutual adjustment and the close adherence of the microspores the evidences of grouping are lost.

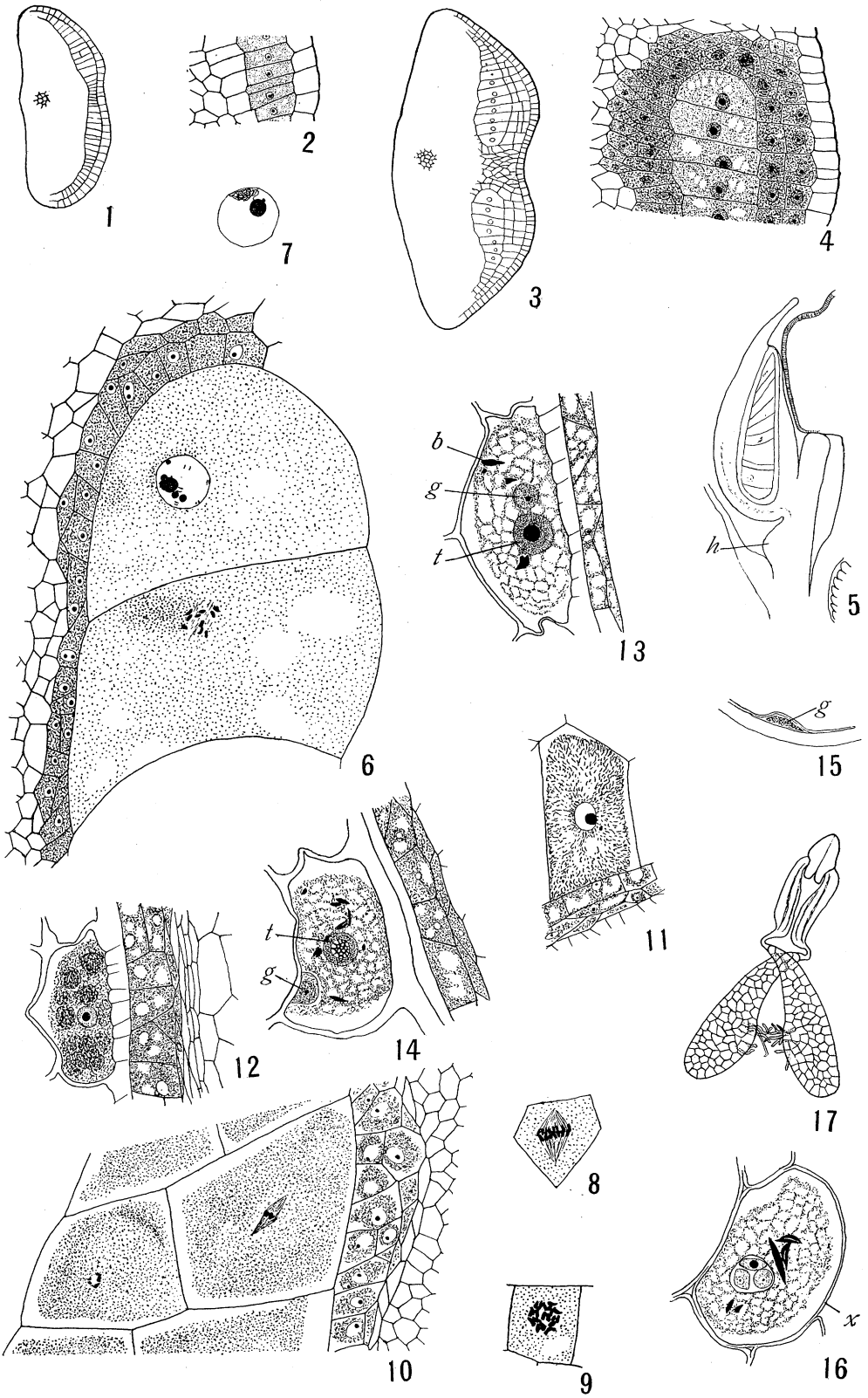
I wish to acknowledge my indebtedness to Dr. John M. Coulter and Dr. Charles J. Chamberlain for their kindly direction and valuable suggestions.

HULL BOTANICAL LABORATORY,
THE UNIVERSITY OF CHICAGO.

NOTE.—Just as this paper goes to press, one by Professor Strasburger, *Einige Bemerkungen zu Pollenbildung bei Asclepias* (Ber. deutsch. bot. Gesells. 19: 450–454. 1901), dealing with *Asclepias Cornuti* and *Cynanchum Vincetoxicum*, has come to hand, confirming in a general way the results reported above.

EXPLANATION OF PLATE XIII.

The figures are reduced to one half their original size. The lenses used were Leitz objectives 3 and 7, and Zeiss $\frac{1}{12}$ oil immersion; oculars, Reichert



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1 and 2, and Zeiss compensating 4, 8, 12, and 18. All drawings were sketched with a Bausch and Lomb camera lucida.

Figs. 1–4. Asclepias Cornuti.

FIG. 1. Cross section of young stamen showing archesporium just beginning the division into primary wall and primary sporogenous layers; somewhat diagrammatic. × 290.

FIG. 2. Part of *fig. 1*, through archesporium. × 800.

FIG. 3. Section across a stamen showing primary sporogenous cells grading into vegetative cells and division of primary wall layer into four layers; partly diagrammatic. × 290.

FIG. 4. Part of *fig. 3*, showing tapetal nature of cells surrounding the primary sporogenous cells. × 800.

Figs. 5–16. Asclepias tuberosa.

FIG. 5. Longitudinal section through stamen showing location of sporangium and orientation of cells within; *h*, hood; partly diagrammatic. × 22.

FIG. 6. Longitudinal section of lower part of sporangium; two spore mother cells undergoing first division; one nucleus in aster stage, the other with chromosomes just split. × 800.

FIG. 7. Synapsis stage of nucleus in spore mother cell. × 800.

FIG. 8. Aster stage in division of a vegetative cell. × 2150.

FIG. 9. Axial view of plate of chromosomes in aster stage in vegetative cell. × 2150.

FIG. 10. Longitudinal section of sporangium near its top showing second division of nucleus of spore mother cell. × 800.

FIG. 11. Section of young microspore showing thready protoplasm; tapetal cells adjacent. × 800.

FIG. 12. Section of microspore containing masses of denser ptotoplasm; walls already thickened; tapetum adjacent. × 800.

FIG. 13. Section of microspore; *b*, deeply staining bodies; *g*, generative nucleus; *t*, tube nucleus.

FIG. 14. Section of microspore showing generative nucleus (*g*) flattened against the wall of the cell and just cut off. × 800.

FIG. 15. Section of a generative cell cut off from the rest of the microspore by a considerable wall. × 900.

FIG. 16. Section of microspore containing what may be a fragmented tube nucleus; *x*, side torn from pollinium wall. × 800.

Fig. 17. Asclepias incarnata.

FIG. 17. Sketch of two pollinia germinating in a 10 per cent. solution of cane sugar; partly diagrammatic. × 45.